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Levels of Lead in Breast Milk and Their Relation to Maternal Blood and Bone Lead Levels at One Month Postpartum

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Despite the many well-recognized benefits of breast-feeding for both mothers and infants, detectable levels of lead in breast milk have been documented in population studies of women with no current environmental or occupational exposures. Mobilization of maternal bone lead stores has been suggested as a potential endogenous source of lead in breast milk. We measured lead in breast milk to quantify the relation between maternal blood and bone lead levels and breast-feeding status (exclusive vs. partial) among 310 lactating women in Mexico City, Mexico, at 1 month postpartum. Umbilical cord and maternal blood samples were collected at delivery. Maternal breast milk, blood, and bone lead levels were obtained at 1 month postpartum. Levels of lead in breast milk ranged from 0.21 to 8.02 µg/L (ppb), with a geometric mean (GM) of 1.1 µg/L; blood lead ranged from 1.8 to 29.9 µg/dL (GM = 8.4 µg/dL); bone lead ranged from < 1 to 67.2 µg/g bone mineral (patella) and from < 1 to 76.6 µg/g bone mineral (tibia) at 1 month postpartum. Breast milk lead was significantly correlated with umbilical cord lead [Spearman correlation coefficient (r_s) = 0.36, $p < 0.0001$] and maternal blood lead (r_s = 0.38, $p < 0.0001$) at delivery and with maternal blood lead (r_s = 0.42, $p < 0.0001$) and patella lead (r_s = 0.15, $p < 0.01$) at 1 month postpartum. Mother's age, years living in Mexico City, and use of lead-glazed ceramics, all predictive of cumulative lead exposure, were not significant predictors of breast milk lead levels. Adjusting for parity, daily dietary calcium intake (milligrams), infant weight change (grams), and breast-feeding status (exclusive or partial lactation), the estimated effect of an interquartile range (IQR) increase in blood lead (5.0 µg/dL) was associated with a 33% increase in breast milk lead [95% confidence interval (CI), 24 to 43%], whereas an IQR increase in patella lead (20 µg/g) was associated with a 14% increase in breast milk lead (95% CI, 5 to 25%). An IQR increase in tibia lead (12.0 µg/g) was associated with a 5% increase in breast milk lead (95% CI, -3% to 14%). Our results indicate that even among a population of women with relatively high lifetime exposure to lead, levels of lead in breast milk are low, influenced both by current lead exposure and by redistribution of bone lead accumulated from past environmental exposures. **Key words:** blood lead, breast milk lead, breast-feeding, KXRF bone lead, lactation. *Environ Health Perspect* 112:926–931 (2004). doi:10.1289/ehp.6615 available via <http://dx.doi.org/> [Online 7 April 2004]

Although substantial attention has been given to the risks for the developing fetus from circulating lead in maternal blood, much less consideration has been given to the presence of lead in breast milk. There are no clear guidelines regarding breast-feeding to provide counseling to women with elevated blood or bone lead levels (Sinks and Jackson 1999). Previous research has shown that maternal bone lead stores, accumulated from past environmental exposures, are mobilized to a marked degree during pregnancy and lactation (Gulson et al. 1997 1998b; Hernández-Avila et al. 1996; Hertz-Picciotto et al. 2000; Hu et al. 1996; Rothenberg et al. 2000). Breast-feeding practices and maternal bone lead levels have been shown to be important predictors of maternal blood lead levels over the course of lactation (Téllez-Rojo et al. 2002). However, there is less information available about the transfer of lead to breast milk.

Lactation requires a substantial redistribution of maternal calcium that is marked by

mobilization of calcium from bone stores (Sowers 1996). It is estimated that up to 5% of bone mass is mobilized during lactation (Hayslip et al. 1989; Sowers 1996); thus, lead accumulated in bone from past exposures may be released into blood and excreted into breast milk. Generally, there is a low potential for transfer of lead through milk when current maternal exposure levels are low. However, because > 90% of lead in the adult human body is stored in bone (Barry 1975; Barry and Mossman 1970), the possibility exists for significant redistribution of cumulative lead stores from bone into plasma and subsequently into breast milk during periods of heightened bone turnover (e.g., pregnancy and lactation; Silbergeld 1991). This phenomenon constitutes a potential public health problem in areas where environmental lead exposure is continuing as well as in areas where environmental lead exposure has recently declined.

Studies of lead in human breast milk have found concentrations ranging over three orders

of magnitude from < 1 to > 100 µg/L (ppb; Gulson et al. 1998a; Namihira et al. 1993). These differences in lead levels are probably attributable in part to true differences in exposure distributions across populations and over time. Methodologic factors that may affect the reported results include the high potential for contamination of samples, inaccuracy of laboratory analytic methods, and study design issues such as inconsistent sampling and analysis protocols, incomplete reporting of sampling methods, nonrepresentative sampling (geographic, age, parity), timing and duration of sampling, and small numbers of study subjects (LaKind et al. 2001).

Breast milk lead levels have been found to be higher in urban compared to rural populations (Ong et al. 1985); however, it is not possible to distinguish how much of these elevations derives from ongoing environmental exposure as opposed to mobilization of bone lead stores. Some investigators have found that lead levels in breast milk were higher than levels in plasma (Wolff 1983). Bonithon-Kopp et al. (1986) found that women > 30 years of age had significantly higher levels of breast milk lead than did women between 20 and 30 years of age. Because bone accumulates lead with age, the implication was that increased bone lead levels lead to increased breast milk lead levels during lactation.

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By examining the lead isotopic ratios in a small number of postpartum recent immigrants to Australia (and postpartum Australian controls), Gulson et al. (1998a) concluded that the major sources of lead in breast milk are from maternal bone and diet. In addition, they found that the mobilization of lead from bone during lactation continued postpartum for up to 6 months and was larger than that experienced during pregnancy (Gulson et al. 1998b).

We measured breast milk lead levels in a large cohort of lactating women in Mexico City at 1 month postpartum and quantified the relation to maternal blood and bone lead levels and breast-feeding status (exclusive vs. partial). We used a rigorous, well-validated technique to collect, prepare, and analyze the samples of breast milk in this study, thus limiting the potential for contamination and maximizing the percentage recovery of lead from milk samples.

Materials and Methods

We conducted a cross-sectional study of 310 lactating women at 1 month postpartum. Subjects were a subcohort of women recruited for later participation in a randomized placebo-controlled trial of calcium supplementation during lactation. Informed consent, questionnaire information, and samples for the present study were obtained before the initiation of calcium supplementation. All participating mothers received a detailed explanation of the study and counseling on reduction of lead exposure. The research protocol was approved by the human subjects committees of the National Institute of Public Health of Mexico, Harvard School of Public Health, and the participating hospitals.

Data collection methods have been described in detail elsewhere (Hernández-Avila et al. 2003). Between January 1994 and June 1995, 2,945 potential study participants were interviewed at three maternity hospitals in Mexico City. Of these, 1,398 were eligible for the trial. Exclusion criteria included logistics that would interfere with data collection, such as living outside of Mexico City; physician's diagnosis of multiple fetuses, pre-eclampsia, or pregnancy-related hypertension; psychiatric, kidney, or cardiac disease; gestational diabetes; history of repeated urinary infections; family or personal history of kidney stone formation; seizure disorder requiring daily medications; and ingestion of corticosteroids or other factors that may modify calcium metabolism. Women who gave birth to premature infants (< 37 weeks) also were excluded.

Of the women identified as eligible, 629 (45%) agreed to participate in the study. These women completed a baseline evaluation, including questionnaires that assessed known risk factors for environmental lead exposure, including current and past use of lead-glazed

ceramics; occupational, residential, medical, and reproductive histories; and information about intended breast-feeding practices. Maternal dietary intake was assessed at 1 month postpartum using a self-administered, semi-quantitative food-frequency questionnaire designed to estimate usual dietary intake over an extended period of time (over the course of pregnancy) before completion of the questionnaire. The questionnaire was translated and validated for use in Spanish-speaking populations specifically for the Mexican adult population (Hernández-Avila et al. 1998).

For women identified before delivery, umbilical cord and maternal blood samples were collected at the birth. At 1 month postpartum (± 5 days), field personnel visited study participants at home to obtain anthropometric measurements and blood and breast milk samples. Maternal bone lead was estimated by K-X-ray fluorescence (KXRF) at the research facility at the American British Cowdray (ABC) Hospital. The present analysis is limited to data from 310 subjects with breast milk samples collected at 1 month postpartum with adequate volume for analysis remaining after the pilot phase of the study investigating methods for improved digestion procedures.

Blood lead. Blood lead measurements were performed using graphite furnace atomic absorption spectrophotometry (model 3000; Perkin-Elmer, Norwalk, CT, USA) at the ABC Hospital Trace Metal Laboratory according to a technique described by Miller et al. (1987). The laboratory participates in the Centers for Disease Control and Prevention blood lead proficiency testing program administered by the Wisconsin State Laboratory of Hygiene (Madison, WI, USA), which provided external quality control (QC) specimens varying from 2 to 88 $\mu\text{g}/\text{dL}$. Our laboratory maintained acceptable precision and accuracy over the study period [correlation = 0.98; mean difference = 0.71 $\mu\text{g}/\text{dL}$; SD = 0.68].

Bone lead. We used a spot-source ^{109}Cd KXRF instrument constructed at Harvard University and installed at the research facility in Mexico City to measure maternal bone lead. Thirty-minute *in vivo* measurements of each subject's mid-tibial shaft (representing cortical bone) and patella (trabecular bone) were obtained after each region had been washed with a 50% solution of isopropyl alcohol. The physical principles, technical specifications, validation, and use of the KXRF technique have been described in detail elsewhere (Hu et al. 1991). In brief, the instrument uses a cadmium γ -ray source to provoke the emission of fluorescent photons from target tissue that are then detected, counted, and arrayed on a spectrum. A net lead signal is determined after subtraction of Compton background counts by a linear

least-squares algorithm. The lead fluorescent signal is then normalized to the elastic or coherently scattered γ -ray signal, which arises predominantly from the calcium and phosphorus present in bone mineral (units of measurement in micrograms of lead per gram of bone mineral). The instrument also provides an estimate of the uncertainty associated with each measurement. For QC, we excluded bone lead measurements with uncertainty estimates that were > 10 and $15 \mu\text{g Pb/g}$ mineral bone for tibia ($n = 12$) and patella ($n = 38$), respectively, from the entire cohort of 629 women. These high uncertainty measurements generally reflect excessive patient movement outside of the measurement field or excessive thickness of overlying tissue and do not produce acceptable results.

Breast milk lead. Breast milk samples were collected at 1 month postpartum from lactating women. Samples were obtained and analyzed using techniques to minimize potential for environmental contamination and to determine lead concentrations in breast milk with a high percentage of recovery. Before manually expressing milk, the breast was washed with deionized water that also was collected and analyzed for lead contamination. Ten milliliters of milk was collected in preleached polypropylene tubes. Samples were frozen, shipped to the Channing Laboratory (Boston, MA, USA), and stored at -30°C (Fisher IsoTempPlus) until analysis.

In pilot work, three different digestion procedures for trace lead analysis of human breast milk were evaluated: *a*) ashing acid-pretreated samples in a muffle furnace, *b*) microwave digestion with nitric acid (HNO_3) using PAAR bombs—a microwave acid digestion bomb used for the dissolution of analytical samples (Anton Paar USA, Ashland, VA, USA), and *c*) digestion with HNO_3 in a high-temperature high-pressure asher (HPA; Anton Paar USA, Ashland, VA, USA). Digestion with HNO_3 in an HPA was chosen as the preferred method because it gave the most accurate and reproducible results with very low blanks. All sample handling was performed under a class-100 clean hood. QC samples were included from National Institute of Standards and Technology (NIST) standard reference materials (SRMs) with certified lead concentrations (NIST SRM 1549 nonfat milk and NIST SRM 8439 whole milk powder).

Breast milk samples were mixed thoroughly for 15 min in an ultrasonic mixer (sonicator) before aliquoting for the analysis. A 2-g aliquot of breast milk, 0.5 g of isotope dilution spike (a solution of 10 ng/mL NIST SRM 983, ^{206}Pb -enriched), and 1 mL HNO_3 (Optima grade, Fisher Chemicals, Fairlawn, NJ, USA) were digested in a sealed 35-mL capacity quartz vessel in the HPA. Five such vessels were inserted in an aluminum heater

block and placed in an autoclave unit. The unit was pressurized with nitrogen under high pressure (115–118 bar), and the samples were heated at 230°C for 90 min under computer control with a sequenced temperature-time program using a modified method of Amarasiwardena et al. (1997). Two sets of five samples were digested each day. Each batch contained one method blank. After digestion, samples were cooled to room temperature, and the resulting solution was transferred to 15-mL plastic tubes and diluted to 15 mL with distilled deionized water.

Lead content in the samples was analyzed by isotope dilution-inductively coupled plasma mass spectrometry (ID-ICPMS; Sciex Elan 5000; Perkin-Elmer). A batch of 24 samples contained one blank, two QC standards, one spiked sample, and one duplicate sample. The limit of detection for lead analysis in breast milk by HPA digestion and ID-ICPMS is 0.1 ng/mL (ppb) milk. The accuracy of spiked samples and QC standards at this concentration level (1 ng/mL) is > 90%, and precision of the measurement is < 5% relative standard deviation (RSD). Sample preparation was performed at University Research Institute for Analytical Chemistry (Amherst, MA, USA), and instrumental analysis was performed at the Trace Metals Laboratory of Harvard School of Public Health.

Statistical analysis. Univariate and bivariate summary statistics and distributional plots were examined for all variables. Characteristics

of the participants with and without breast milk lead levels available were compared using Wilcoxon/chi-squared tests of equality of sample means/proportions. Breast milk lead levels were highly positively skewed. Ten extreme outliers were identified using the generalized extreme studentized deviation many-outlier procedure (Rosner 1983) and were excluded from the multivariate regression analyses. The log(base *e*)-transformed values of the dependent variable were used. Possible associations between breast milk lead and the independent variables were explored separately with bivariate linear regression models. Spearman tests of correlation were used, and correlation coefficients with *p*-values are reported. Nonparametric smoothing (Lowess; bandwidth = 0.75) was used to describe the associations between the different lead biomarkers. Multiple linear regression models were used to describe the relationships between breast milk and the covariates of interest, which were determined *a priori* based on biologic considerations. Parity, daily dietary calcium intake (milligrams), breast-feeding status (exclusive vs. partial), and infant weight change (as a surrogate for the amount of breast milk consumed) were included along with each of the lead exposure variables in separate models. Because of the different units of measurement for the blood and bone lead biomarkers, direct comparison of the regression coefficients is not possible. To compare the effect estimates for each of the maternal lead biomarkers on breast milk lead, we estimated the effect of interquartile range (IQR)

increase in the maternal lead biomarker on log breast milk lead (μg/L) at 1 month postpartum, expressed as percent change with 95% confidence intervals (95% CIs). All statistical analyses were performed using Statistical Analysis System (SAS) software (release 8.01; SAS Institute, Inc., Cary, NC, USA) and S-PLUS (6.0 Professional Edition for Windows; Insightful Corp., Seattle, WA, USA).

Results

On average, women in our Mexico City cohort (*n* = 629) were 24.5 years of age (range, 14–44 years) and had lived in Mexico City for 20.5 years (range, 0.5–44 years); 43% were primiparous. Of the 358 women with prior pregnancies, 32% (*n* = 116) had completed 12 or more months of total breast-feeding of their previous infants. Women with breast milk lead measurements available were more likely to have been breast-feeding exclusively at 1 month postpartum (*p* < 0.0001) and reported more total months of breast-feeding in previous pregnancies (*p* < 0.0432) than did women in the cohort without breast milk samples available for analysis (Table 1). Because early pilot work degraded the milk samples provided by some individuals, subjects with milk available for additional chemical analyses tended to have provided larger volumes of milk and therefore were more likely to have been exclusively breast-feeding. In addition, they were more likely to be married (*p* = 0.0038) and had a slightly higher average estimated daily calcium intake (*p* = 0.005) than women without breast milk lead levels available.

Figure 1 shows the distribution of breast milk lead concentrations (*n* = 310) at 1 month postpartum. The breast milk lead levels were highly positively skewed. Levels of lead in breast milk ranged from 0.21 to 8.02 μg/L (ppb), with a geometric mean (GM) of 1.1 μg/L.

Breast milk lead was significantly correlated with maternal blood [Spearman correlation coefficient (*r_s*) = 0.42, *p* < 0.0001] and maternal patella lead (*r_s* = 0.15, *p* < 0.01) at 1 month postpartum (Figure 2). Breast milk lead was also significantly correlated with umbilical cord (*r_s* = 0.36, *p* < 0.0001) and

Table 1. Differences in subject characteristics between participants with (*n* = 310) and without (*n* = 319) breast milk lead measurements available at 1 month postpartum.

Subject characteristics	With breast milk		Without breast milk		<i>p</i> -Value ^a
	No.	Mean ± SD (range)	No.	Mean ± SD (range)	
Age of mother (years)	308	24.4 ± 5.0 (14–43)	315	24.6 ± 5.3 (15–44)	0.58
Years living in Mexico City	308	20.1 ± 8.7 (0.5–40)	315	20.9 ± 7.9 (1–44)	0.22
No. of years of school	304	9.3 ± 3.1 (1–18)	310	9.4 ± 3.0 (1–17)	0.75
Married (%)	308	70.1	315	59.1	0.004
No. of prior pregnancies ^b	308	2.0 ± 1.2 (1–7)	315	2.0 ± 1.3 (1–8)	0.90
Primiparity (%)	308	41.9	315	45.1	0.42
Previous lactation > 12 months (%)	310	21.6	319	15.4	0.04
Exclusive breast-feeding (%)	308	32.1	314	14.3	< 0.0001
Estimated daily calcium intake (mg)	309	1,138 ± 390 (93–2,532)	319	1,072 ± 414 (108–2,751)	0.04
Current use of lead-glazed ceramics (%)	310	40.7	319	39.8	0.83
Past use of lead-glazed ceramics (%)	308	76.6	315	78.4	0.59
Current smoking or during pregnancy (%)	310	6.1	319	7.5	0.49
Maternal blood lead (μg/dL)	310	9.3 ± 4.4 (1.8–29.9)	319	9.3 ± 4.9 (2.3–60.1)	0.98
Maternal patella lead (μg/g bone mineral) ^c	294	14.5 ± 14.9 (< 1–67.2)	289	15.2 ± 16.1 (< 1–85.9)	0.58
Maternal tibia lead (μg/g bone mineral) ^c	303	9.6 ± 10.1 (< 1–76.5)	306	10.5 ± 10.2 (< 1–51.0)	0.31

^a*p*-Value from *t*-test of equality of two sample population means or chi-square test of equality of two sample proportions.

^bIncluding the index pregnancy (primiparous women = 43.1%). ^cIncludes measurements with negative values: patella (*n* = 134), tibia (*n* = 99).

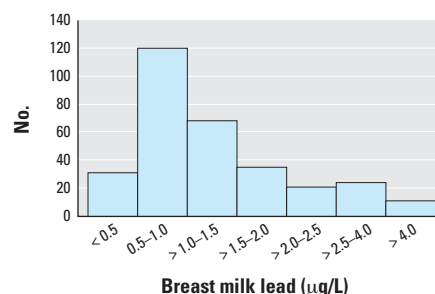


Figure 1. Distribution of breast milk lead levels (μg/L) at 1 month postpartum (*n* = 310).

maternal blood ($r_s = 0.38$, $p < 0.0001$) lead at delivery. Figure 3 displays a plot of maternal blood lead ($\mu\text{g/L}$) by the ratio of breast milk lead to maternal blood lead ($\times 100$) at 1 month postpartum. Breast milk lead expressed as percentage of maternal blood lead ranged from 0.4 to 9.2% (mean = 1.6%, SD = 1.2%).

Differences in lead biomarkers by maternal characteristics at 1 month postpartum are shown in Table 2. Breast milk lead levels (mean micrograms per liter \pm SD) were similar among women who reported practicing exclusive breast-feeding (1.38 ± 1.10) compared with women who practiced partial lactation (1.35 ± 1.09). The older women had breast milk lead levels (1.11 ± 0.65) lower than those in the younger age category (1.20 ± 0.83) and lower than women in the 20–30 year age range (1.44 ± 1.19), who comprised most participants. As expected, both patella and tibia bone lead levels increased with age. Mean breast milk lead levels were lower for primiparous women (1.34 ± 1.12) than for women with one previous pregnancy (1.42 ± 1.14) but lower still for women with two or more previous pregnancies (1.30 ± 0.98), although these differences were not statistically significant. For women who had breast-fed previously for 12 months or more (1.31 ± 1.27), breast milk lead levels were slightly lower than for those women who had breast-fed previously for less than 12 months (1.38 ± 0.90) or not at all (1.38 ± 1.22), although these differences were also not statistically significant. Women who reported daily intake of calcium $< 1,000$ mg had higher, though not significantly different ($p = 0.23$), breast milk lead levels (1.45 ± 1.19) than did those who received more adequate amounts of calcium ($\geq 1,000$ mg) in their diets (1.30 ± 1.02). Significant differences in breast milk, blood, and patella lead levels were observed between women who reported current use of lead-glazed ceramics and those who did not use leaded ceramics at the time of the interview. Among women who reported current use of lead-glazed ceramics, breast milk lead levels averaged 1.53 ± 1.17 compared with 1.26 ± 1.03 for those who did not use leaded ceramics ($p = 0.03$).

Mother's age, years living in Mexico City, and use of lead-glazed ceramics, all predictive of cumulative lead exposure, were not significant bivariate predictors of breast milk lead levels. Maternal blood lead at delivery ($\beta = 0.0608$, $p < 0.0001$) and at 1 month postpartum ($\beta = 0.0588$, $p < 0.0001$) were significant predictors of breast milk lead at 1 month postpartum. After adjusting for infant weight change (grams), parity, daily dietary calcium intake, and breast-feeding status (exclusive or partial lactation), patella lead was a significant predictor of breast milk lead ($\beta = 0.0068$, $p = 0.003$). However, patella lead was no longer significant when we controlled for maternal blood lead because the effect of patella bone lead is mediated through blood lead. Maternal blood lead at delivery ($\beta = 0.0396$, $p < 0.0001$) and at 1 month postpartum ($\beta = 0.0363$, $p < 0.0001$) both remained significant independent determinants of lead in breast milk in the multivariate analyses. The estimated effect of an IQR increase in blood lead ($5 \mu\text{g/dL}$) was associated with a 33% increase in breast milk lead (95% CI, 24 to 43%), whereas an IQR increase in patella lead ($20 \mu\text{g/g}$) was associated with a 14% increase in breast milk lead (95% CI, 5 to

25%; Table 3). An IQR increase in tibia lead ($12 \mu\text{g/g}$) was associated with a 5% increase in breast milk lead (95% CI, –3 to 14%).

Discussion

Despite the many well-recognized benefits of breast-feeding for both mothers and infants, detectable levels of lead in breast milk have been documented in population studies of women with no current environmental or occupational exposures (Abadin et al. 1997). Because lead accumulates in bone, women who were chronically exposed to environmental lead during infancy and adolescence may have a significant bone lead burden when they reach reproductive age. We have demonstrated here that maternal blood and bone lead levels are important determinants of lead in breast milk at 1 month postpartum. Breast milk lead levels are influenced both by current environmental exposures and by the redistribution of previously accumulated maternal lead due to bone resorption associated with pregnancy and lactation.

Osterloh and Kelly (1999) assessed lactating women prospectively to study the effect of lactational bone loss on blood lead concentrations

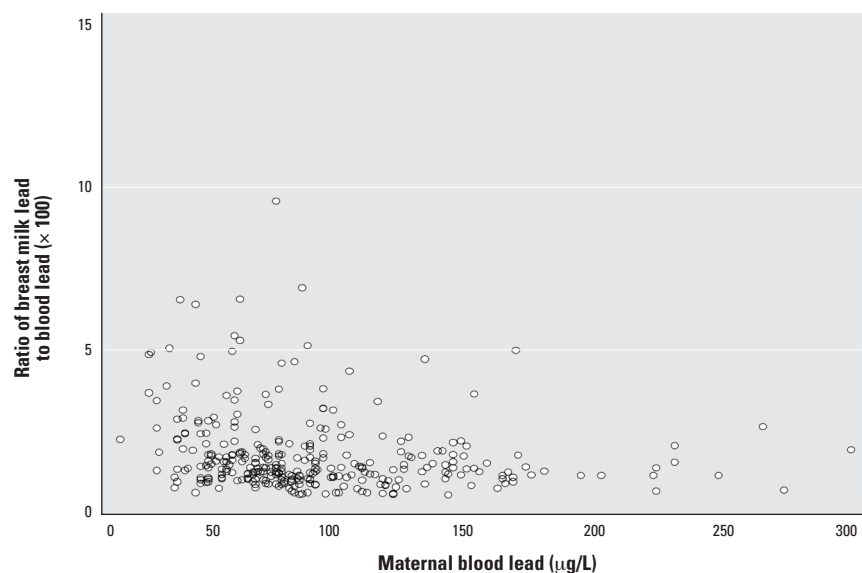


Figure 3. Plot of maternal blood lead ($\mu\text{g/L}$) by ratio of breast milk lead to maternal blood lead ($\times 100$) at 1 month postpartum.

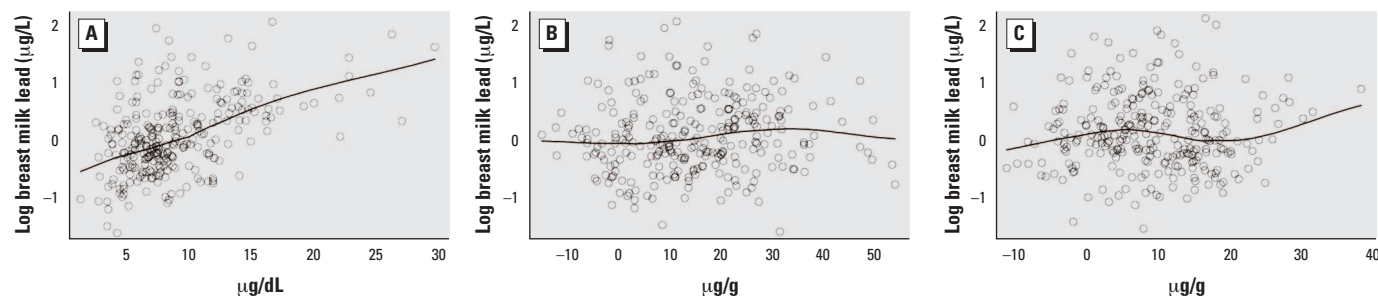


Figure 2. Smooth scatterplots (Lowess; bandwidth = 0.75) of breast milk lead by maternal lead biomarkers at 1 month postpartum: (A) blood lead; (B) patella lead; (C) tibia lead. Breast milk lead levels log(base-e) transformed.

and found that bone density losses averaged 2.5% at the vertebral spine and almost 1% at the femoral neck. The total number of breast-feedings was the only significant independent predictor of final bone density apart from the initial bone density. No changes in blood lead concentration, however, were observed after 2 weeks postpartum. In a study of community volunteers, Kosnett et al. (1994) found that women with a history of breast-feeding had lower age-adjusted tibia bone lead levels than did those without a history of lactation, suggesting that lactation depletes maternal bone lead. Moline et al. (2000) noted a significant inverse relationship between months of lactation and age-adjusted calcaneus lead among 24 lactating women in Mexico City. Hernández-Avila et al. (1996) demonstrated that patellar lead is a

main determinant of circulating blood lead during lactation. A 34- $\mu\text{g/g}$ increase in patella lead (from the medians of the lowest to the highest quartiles) was associated with an increase in blood lead of 2.4 $\mu\text{g/dL}$. Sowers et al. (2002) found direct evidence of bone loss among breast-feeding mothers compared with bottle-feeders by examining changes in bone mineral density and serum osteocalcin levels in relation to maternal blood and breast milk lead levels.

Our results indicate, however, that even among a population of women with relatively high cumulative lifetime exposures to lead, as evidenced by their bone lead levels, levels of lead in breast milk are low. Previous results from this cohort showed that breast-feeding practices and maternal bone lead levels were important predictors of maternal blood lead

levels over the course of lactation (Téllez-Rojó et al. 2002). Here we present levels of lead in breast milk having used a rigorous, well-validated technique to collect, prepare, and analyze the samples. Breast milk lead levels from previous published studies with extremely high values should be reviewed with caution because of the high potential for contamination and inaccuracy of the laboratory analytic methods.

Documented sources of lead contamination in breast milk include use of lead acetate ointment (Knowles 1974), lead in nipple shields (Knowles 1974; Newman 1997), and foil from alcohol wipes used in sample collection (Hu et al. 1996). Because the concentrations of lead in breast milk are low, the influence of environmental contamination is high (Smith et al. 1998).

Gulson et al. (1998a) proposed using the comparison of ratios expressed as percentage of lead concentrations in breast milk to whole blood as a method to verify the results, suggesting that data with a ratio of $> 15\%$ should be treated with caution. High lead concentrations in breast milk relative to blood lead concentrations would thus be an indicator of contamination during sample collection and/or laboratory analysis. In all of the 310 sample pairs in our study, ratios of breast milk to whole blood lead levels were $< 15\%$.

In addition, measurement of lead in breast milk is complicated by the fat content of human milk. The fat content of human breast milk changes during feeding and over the course of lactation (Sim and McNeil 1992). Precise and accurate analysis is challenging because of difficulty in identifying a method that will digest samples with 100% efficiency. Our group has developed these techniques both to obtain breast milk samples that minimize potential for contamination and to determine lead concentrations in breast milk with a high percentage of recovery (Amarasiriwardena C, Hu H, unpublished data). In the technique used for this analysis, we determined the partitioning of lead between the acid soluble and "fat" residue. Lead concentrations in the fat portion alone were close to the detection limit, indicating a high recovery of lead in the milk.

This is a cross-sectional analysis and cannot evaluate changes in breast milk and bone lead

Table 2. Lead concentrations by subject characteristics at 1 month postpartum ($n = 310$).

Subject characteristic	No.	Mean maternal lead concentration \pm SD			
		Breast milk ($\mu\text{g/L}$)	Blood ($\mu\text{g/dL}$)	Patella ($\mu\text{g/g}$)	Tibia ($\mu\text{g/g}$)
Mother's age (years) ^a					
< 20	49	1.20 \pm 0.83	9.4 \pm 4.5	11.6 \pm 12.8	7.4 \pm 7.1
20–30	220	1.44 \pm 1.19	9.3 \pm 4.5	14.7 \pm 15.2	9.6 \pm 10.7
> 30	39	1.11 \pm 0.65	9.0 \pm 3.9	17.6 \pm 15.4	12.4 \pm 9.6
Years spent living in Mexico City ^b					
< 5	26	0.99 \pm 0.43	7.6 \pm 3.1	10.5 \pm 11.7*	5.1 \pm 8.0*
5–20	82	1.29 \pm 1.03	8.9 \pm 4.6	11.2 \pm 13.5	7.7 \pm 10.6
> 20	200	1.43 \pm 1.17	9.7 \pm 4.5	16.5 \pm 15.5	11.0 \pm 10.0
No. of prior pregnancies ^c					
0	129	1.34 \pm 1.12	9.5 \pm 4.7	13.7 \pm 13.6	9.2 \pm 9.2
1	104	1.42 \pm 1.14	8.5 \pm 3.8	14.3 \pm 16.1	9.0 \pm 9.7
≥ 2	75	1.30 \pm 0.98	10.0 \pm 4.7	16.6 \pm 15.2	11.2 \pm 12.1
Previous lactation					
None	104	1.38 \pm 1.22	9.6 \pm 4.8	14.0 \pm 13.5	9.5 \pm 9.6
< 12 months	139	1.39 \pm 0.90	9.3 \pm 4.2	14.9 \pm 15.9	9.9 \pm 10.8
≥ 12 months	67	1.31 \pm 1.27	8.5 \pm 4.3	14.5 \pm 15.1	9.2 \pm 9.4
Reported breast-feeding practice ^d					
Exclusive	99	1.38 \pm 1.10	9.5 \pm 5.0	14.8 \pm 13.2	9.7 \pm 9.3
Partial	209	1.35 \pm 1.09	9.2 \pm 4.1	14.5 \pm 15.7	9.6 \pm 10.5
Current use of lead-glazed ceramics					
Yes	126	1.53 \pm 1.17*	10.8 \pm 5.2*	17.0 \pm 15.1*	10.8 \pm 12.0
No	184	1.26 \pm 1.03	8.2 \pm 3.4	12.8 \pm 14.5	8.8 \pm 8.5
Past use of lead-glazed ceramics ^e					
Yes	236	1.40 \pm 1.11	9.3 \pm 4.3	14.5 \pm 15.2	10.1 \pm 10.5
No	72	1.23 \pm 1.03	9.3 \pm 4.8	14.7 \pm 14.0	8.3 \pm 8.8
Dietary calcium intake ^f					
< 1,000 mg	138	1.45 \pm 1.19	9.5 \pm 4.6	13.4 \pm 15.3	10.3 \pm 10.2
$\geq 1,000$ mg	171	1.30 \pm 1.02	9.0 \pm 4.3	15.2 \pm 14.5	9.1 \pm 10.1

^aTwo subjects missing information on mother's age. ^bTwo subjects missing information on years spent living in Mexico City. ^cTwo subjects missing information on number of prior pregnancies. ^dTwo subjects missing information on reported breast-feeding practice. ^eTwo subjects missing information on past use of lead-glazed ceramics. ^fOne subject missing information on dietary calcium intake. *Significantly different at 0.05 level by analysis of variance.

Table 3. Estimated effect^a of IQR increase in maternal lead biomarker on log breast milk lead^b ($\mu\text{g/L}$) at 1 month postpartum expressed as percent change (95% CI).

Predictor variable(s)	Model					
	A	B	C	D	E	F
Blood lead at delivery IQR = 5 $\mu\text{g/dL}$	36 (26 to 46)	—	—	—	22 (12 to 33)	—
Blood lead at 1 month postpartum IQR = 5 $\mu\text{g/dL}$	—	33 (24 to 43)	—	—	20 (10 to 30)	33 (23 to 43)
Patella lead at 1 month postpartum IQR = 20 $\mu\text{g/g}$	—	—	14 (5 to 25)	—	—	3 (–5 to 13)
Tibia lead at 1 month postpartum IQR = 12 $\mu\text{g/g}$	—	—	—	5 (–3 to 14)	—	—
						–2 (–9 to 5)

^aAdjusted for estimated daily calcium intake (milligrams); infant weight change (grams); number of prior pregnancies; and breast-feeding practice (exclusive vs. partial lactation).

^bBreast milk lead levels log transformed.

levels over the course of lactation. It will also be important to determine whether the degree of this influence changes over the course of lactation. Factors that may modify levels of chemicals in breast milk were only partially controlled and include fat content of breast milk, extent and duration of breast-feeding, maternal age and body weight, duration of lactation, parity, multiple pregnancies, race, socioeconomic status, season, and maternal nutrition and hormone levels. However, this study represents the largest epidemiologic study of lead in breast milk in relation to maternal lead biomarkers and is, to our knowledge, the only study using *in vivo* measurement of lead in bone.

Given that bone lead has a half-life of years to decades, infants will continue to be at risk for exposure from maternal bone lead stores long after environmental sources of lead have been abated. The overall decline of ambient environmental concentrations of lead highlights the importance of bone lead as an endogenous source of exposure. However, given the low levels of lead in breast milk demonstrated here, the contribution from foods and beverages used as alternatives to or in combination with breast milk may be similar to or greater than that of breast milk. All levels were below the current U.S. Environmental Protection Agency (EPA) maximum contaminant level for lead in water (15 ppb; U.S. EPA 1991), which is relevant because the alternative to breast-feeding, infant formula, is often prepared with tap water.

Better understanding of neonatal exposure, including kinetics in the lactating mother and in the newborn, and knowledge about alternative dietary sources of lead are needed for risk assessment. Estimating the potential lead dose to infants from breast milk requires information on the quantity of breast milk consumed per day and the duration over which breast-feeding occurs (U.S. EPA 1997). Additional information on the lead content of dietary alternatives should be investigated in comparison with breast milk levels in a specific population, and interactions with other nutritional factors should also be considered. Despite the potential for lead exposure, breast milk remains the best and most complete nutritional source for young infants, especially given the low

levels of lead in breast milk in this group of mothers with relatively high cumulative lifetime exposures to lead.

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